

Fabrication of carbon coated gadolinia particles for dual-mode magnetic resonance and fluorescence imaging

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Abstract: In the present study, we report a fabrication of dual-mode carbon coated gadolinia C@Gd₂O₃ particles by a facile hydrothermal synthesis method without using any organic solvents. The prepared C@Gd₂O₃ particles have a core-shell structure and a narrow size distribution in the range of 261±27 nm. The fluorescent properties of the prepared C@Gd₂O₃ particles were accessed by a room-temperature photoluminescence study, while the longitudinal relaxivity (r_1) was examined by using a clinical 1.5 T MRI scanner. A murine fibroblast L-929 cell line was used to examine the cytotoxicity and capability of the prepared C@Gd₂O₃ particles for the fluorescent labeling. The obtained results show that the prepared C@Gd₂O₃ particles could be used as a dual-mode contrast agent for magnetic resonance and fluorescence imaging.

Keywords: carbon; core-shell structure; gadolinia particles; fluorescence; magnetic resonance imaging (MRI) contrast agent

1 Introduction

During the last decade, various functional nanoparticles (NPs) with interesting magnetic, optical, and chemical properties have been extensively applied to biomedical areas including imaging, diagnosis, and therapy [1–5]. Among them, “positive” contrast agents (CAs) are widely used to improve the contrast between tissues using T_1 -weighted magnetic resonance imaging (MRI). The higher density of magnetic ions is the main advantage of using paramagnetic gadolinium oxide NPs as a “positive” CA [6]. The resulting gadolinia

NPs covered with biocompatible polyethylene glycol (PEG) have been used in cell labeling studies [6,7]. In particular, thin PEG layer on the surface of Gd₂O₃ enhances the steric repulsion between NPs, and prevents protein adhesion that leads no NPs aggregation [8]. However, leaching of free Gd³⁺ metal ions from these NPs could cause high toxicity towards natural organs and tissues. Therefore, design of new CAs for biomedical application requires a controlled synthesis, with a suitable surface modification in order for them to remain nontoxic and biocompatible. Furthermore, the aqueous dispersion of these CAs should be stable in aqueous media and buffer solutions.

From other point of view, a combination of paramagnetic and fluorescent properties of different materials into a single system might greatly enhance

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their application areas [9]. For example, these bimodal nanostructures can provide an all-in-one diagnostic and therapeutic tool, which can be utilized to treat and visualize diseases simultaneously. Thus, the development of a simple and reliable fabrication method for the synthesis of multifunctional structures with controlled morphologies is still a challenge. In addition, considering that a large dose of CA is required in a daily imaging process, large-scale process to construct multifunctional CA is still highly needed.

Herein, we design and construct multifunctional carbon coated Gd_2O_3 particles. The morphological and structural properties of the synthesized particles were examined using transmission electron microscopy (TEM) and X-ray diffraction (XRD), respectively. As the main focus of this study, the fluorescent and paramagnetic properties of the prepared CA were studied using a photoluminescence machine and a clinical 1.5 T MRI scanner, respectively. From the view of material synthesis, not only the large-scale synthesis but also the surface modification was easily achieved. We believe that a unique particle system composed of Gd_2O_3 core and coated with thin carbon layer would have great potential in both bio- and nanoscience.

2 Experimental

2.1 Sample preparation

Analytical grade Gd_2O_3 (99.9%), HNO_3 (70%), dextrose ($\geq 99.5\%$), and urea (99.0%–100.5%) were purchased from Sigma-Aldrich and used as received. Spherical Gd_2O_3 NPs were fabricated according to the reported protocols [7,10]. First, a sealed beaker with a freshly prepared aqueous solution of gadolinium nitrate (0.001 mol in 40 mL of H_2O) was placed into an electrical furnace and heated to 90 °C for 1.5 h. The dried precipitates were then calcined in air at 800 °C for 1 h to produce the gadolinia particles. Second, a sealed beaker containing the dispersed Gd_2O_3 particles (3 mg) and an aqueous solution of dextrose (4 g in 40 mL of H_2O) was heated to 180 °C for 8 h. The suspension was then cooled to room temperature, followed by dialysis in deionized ultrapure water for 24 h, to eliminate the free Gd^{3+} ions and excess of dextrose. The carbon coated Gd_2O_3 particles were isolated by centrifugation and dried at 60 °C for 1 day.

2.2 Characterization

The structure of the prepared powders was examined by XRD (Bruker D8 Discover) using $\text{Cu K}\alpha$ radiation ($\lambda = 0.15405$ nm) at a 2θ scan range of 20°–60°. The morphology of the samples was characterized by transmission electron microscopy (TEM; Hitachi H-7600). UV–visible (UV–Vis) absorption spectra were analyzed using a UV–Vis spectrophotometer (Evolution 220). Photoluminescence (PL) measurements were performed using a Hitachi F-7000 spectrophotometer equipped with a 150 W xenon lamp as the excitation source. Dynamic light scattering (DLS) size measurements were performed using the Malvern Zetasizer Nano ZS machine. The T_1 -weighted images were obtained using a 1.5 T MRI scanner (General Electric, Signa, HDx1.5T, USA) using the T_1 -weighted spin-echo method (TR/TE = 500 ms/15 ms, field of view (FOV) = 180 mm \times 180 mm, slice thickness = 2 mm, matrix = 256 \times 204, number of excitations (NEX) = 2). All measurements were performed at a room temperature of 22 \pm 1 °C.

2.3 Cell culture and cytotoxicity assay

A murine fibroblast cell line (L-929 cells from subcutaneous connective tissue) was obtained from the American Type Culture Collection (ATCC CCL-1TM, Rockville, MD). The cells were routinely maintained in Dulbecco's modified Eagle's medium (Sigma-Aldrich), supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 1% antibiotic antimycotic solution (including 10000 units penicillin, 10 mg streptomycin, and 25 mg amphotericin B per mL, Sigma-Aldrich) at 37 °C in 95% humidity and 5% CO_2 . The number of viable cells was indirectly quantified using highly water-soluble tetrazolium salt (WST-8, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium, monosodium salt) (Dojindo Lab., Kumamoto, Japan), reduced to a water-soluble formazan dye by mitochondrial dehydrogenases. The cell viability was found to be directly proportional to the metabolic reaction products obtained in WST-8. Briefly, the WST-8 assay was conducted as follows. L-929 cells were treated with increasing concentrations (0–500 ppm) of C@ Gd_2O_3 composite particles and then incubated with WST-8 for the last 4 h of the culture periods (24 h) at 37 °C in the dark. Parallel sets of wells containing freshly cultured nontreated cells were regarded as negative controls.

The absorbance was determined to be 450 nm using an ELISA reader (SpectraMax[®] 340, Molecular Device Co., Sunnyvale, CA). The relative cell viability was determined as the percentage ratio of the optical densities in the medium (containing the composite particles at each concentration) to that of the fresh control medium.

2.4 Fluorescence microscopy

To examine the cellular uptake and distribution of C@Gd₂O₃ composite particles within the L-929 cells and subsequent cell imaging, the cells were treated with 20 ppm of composite particles for 24 h. After treatment, the cells were fixed with 3.5% paraformaldehyde (Sigma-Aldrich) in 0.1 M phosphate buffer (pH=7) for 10 min at room temperature and immediately observed under a fluorescence microscope (IX81-F72, Olympus Optical, Osaka, Japan).

3 Results and discussion

In a typical fabrication procedure, the spherical Gd₂O₃ spheres with an average diameter of 243±38 nm (Fig. 1(a)) are used as the template cores. A facile hydrothermal method is used to form a thin carbon layer on the surface of Gd₂O₃ spheres as shown in Fig. 1(b). The core-shell structure can be clearly observed due to the different electron penetrability of the cores and shells. The cores are black spheres, whereas the shells have a light grey color with a mean thickness of ~17 nm. The average diameters of the samples, as measured by DLS (Fig. 2) before and after carbon coating, are in accordance with the estimated sizes from TEM analysis. These colloidal suspensions are stable for 2–3 days. However, when the mixtures are shaken or ultrasonicated for several seconds, a homogeneous suspension was formed again.

Figure 3 displays the typical XRD patterns of the Gd₂O₃ and C@Gd₂O₃ spheres. All XRD peaks can be assigned easily to the standard cubic Gd₂O₃ structure (JCPDS No. 88-2165), which belongs to the *Ia3* (206) space group with a lattice constant *a*=1.079 nm [11,12]. No additional peaks are observed indicating the formation of a pure cubic Gd₂O₃ cubic phase. However, a broad diffraction peak for amorphous carbon is also observed in the case of C@Gd₂O₃ spheres.

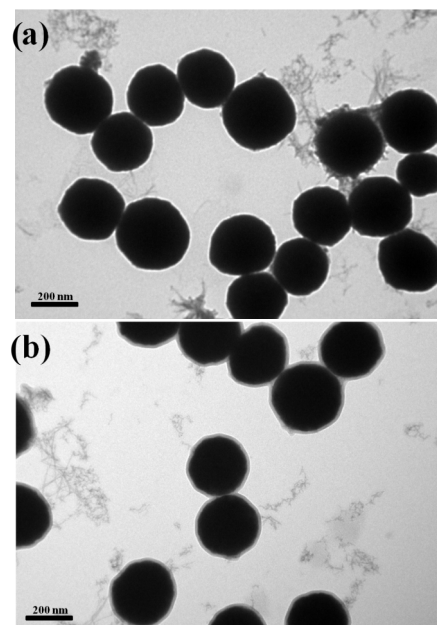


Fig. 1 TEM images of (a) bare Gd₂O₃ spheres and (b) carbon coated Gd₂O₃ spheres.

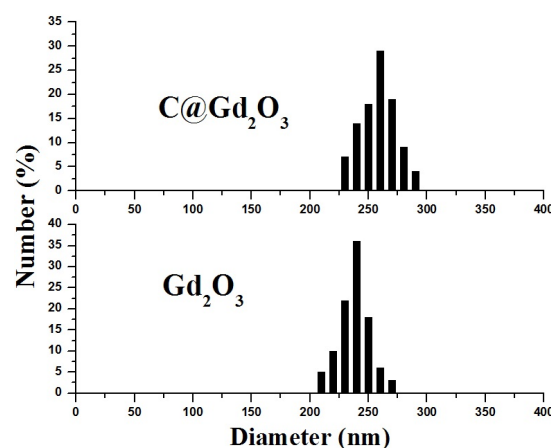


Fig. 2 DLS measurements of the Gd₂O₃ and C@Gd₂O₃ spheres.

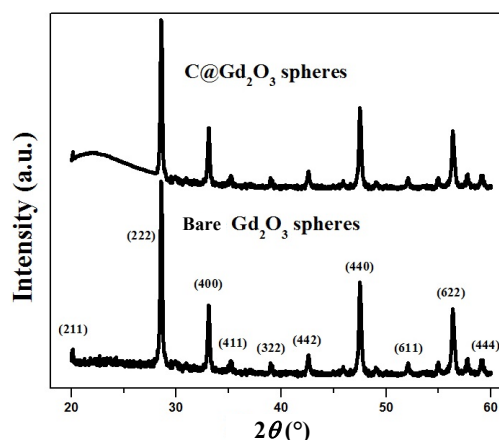


Fig. 3 XRD patterns of bare Gd₂O₃ and carbon coated Gd₂O₃ spheres.

Figure 4 shows the UV–Vis absorbance of the Gd_2O_3 spheres before and after carbon coating. A red shift in absorption peak is observed after carbon coating on the surface of Gd_2O_3 spheres. The absorption of bare Gd_2O_3 spheres starts at wavelengths of about 170 nm, shorter than the absorption of $\text{C@Gd}_2\text{O}_3$ spheres. Resulting $\text{C@Gd}_2\text{O}_3$ spheres possess good optical properties (Fig. 4, inset) with a clearly resolved excitation peak (black line, $\lambda_{\text{max}} = 362$ nm) and an emission peak (red line, $\lambda_{\text{max}} = 451$ nm), which produce bright blue fluorescence. Furthermore, the excitation spectra of $\text{C@Gd}_2\text{O}_3$ spheres are well consistent with the measured UV–Vis absorption spectra. Thus, one can conclude that the surface of the Gd_2O_3 spheres is covered with a thin carbon layer.

To evaluate the cytotoxicity of the prepared samples, L-929 cells were treated with various concentrations of particles for 24 h using a WST-8 assay. Figure 5

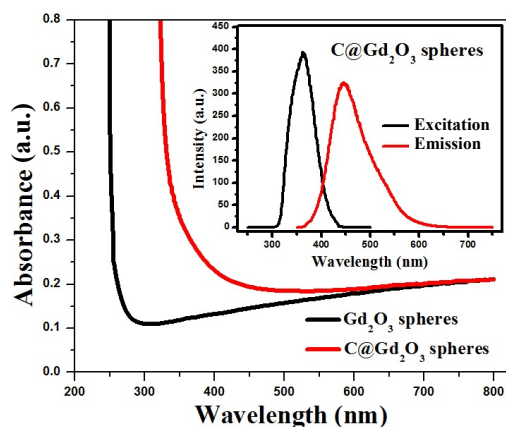


Fig. 4 UV–Vis absorption spectra of the Gd_2O_3 and $\text{C@Gd}_2\text{O}_3$ spheres. Inset shows the PL excitation and emission spectra of $\text{C@Gd}_2\text{O}_3$ spheres.

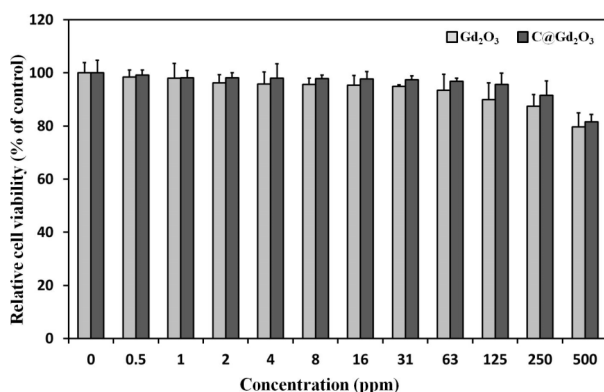


Fig. 5 Dose-dependent responses of L-929 cells to Gd_2O_3 and $\text{C@Gd}_2\text{O}_3$ particles. Relative cell viability of L-929 cells exposed for 24 h to increasing concentrations (0–500 ppm) of samples was evaluated using the WST-8 assay.

shows that the L-929 cells exposed to increasing concentrations (0–500 ppm) of particles for 24 h show a noticeable dose-dependent decrease in their relative cell viability. The bare Gd_2O_3 spheres begin to induce a slight decrease in cell viability from ~ 10 ppm. On the contrary, the $\text{C@Gd}_2\text{O}_3$ particles with a carbon coating result in no significant decrease in cell viability at relatively higher concentrations ~ 30 ppm. These results show that the satisfactory biocompatibility of the $\text{C@Gd}_2\text{O}_3$ particles is at dosages lower than ~ 30 ppm.

To demonstrate the cell imaging potentials of the $\text{C@Gd}_2\text{O}_3$ spheres, the cultured monolayer of L-929 cells was incubated for 24 h with a particle suspension at a concentration of 20 ppm. Figure 6(a) shows that the L-929 cells grow with normal fibroblast-like morphologies after co-labeling with the $\text{C@Gd}_2\text{O}_3$ particles. The obvious blue fluorescence from the $\text{C@Gd}_2\text{O}_3$ particles (Fig. 6(b)) is clearly observed, indicating that the $\text{C@Gd}_2\text{O}_3$ particles could make cell imaging possible through the uniform distribution within the cells.

A 1.5 T clinical MRI scanner was further utilized to demonstrate the potential of the $\text{C@Gd}_2\text{O}_3$ particles for T_1 -weighted MR imaging. Figure 7 shows that the T_1 -relaxation time of the water protons is strongly

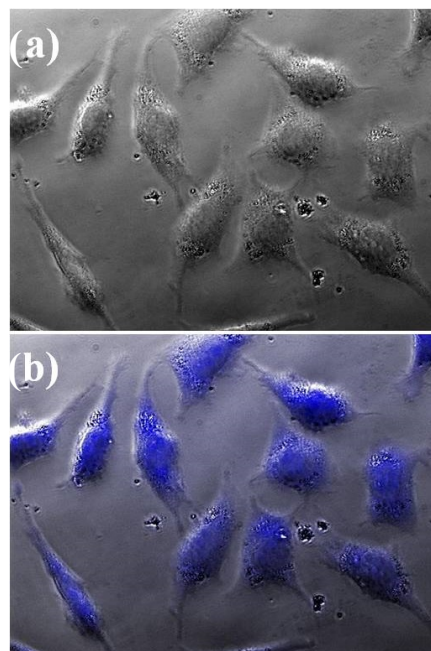


Fig. 6 Fluorescence micrographs (400 \times) of L-929 cells treated with 20 ppm of $\text{C@Gd}_2\text{O}_3$ particles for 24 h. (a) Phase contrast image of the L-929 cells labeled with $\text{C@Gd}_2\text{O}_3$ particles; (b) fluorescence image of the L-929 cells labeled with $\text{C@Gd}_2\text{O}_3$ particles.

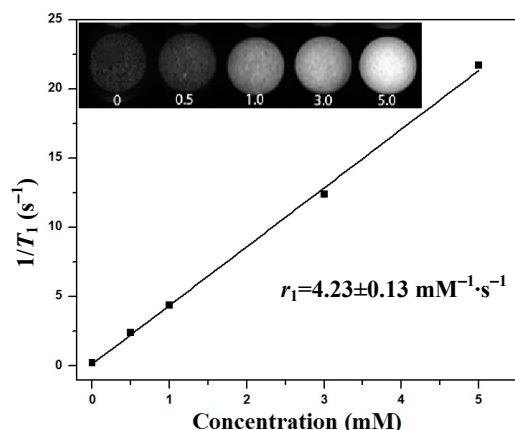


Fig. 7 MRI relaxation rate ($1/T_1$) of C@Gd₂O₃ particles. Inset shows the T_1 -weighted spin-echo MR image of various concentrations of C@Gd₂O₃ particles in water.

shortened and T_1 -weighted images become brighter as the concentration of the C@Gd₂O₃ spheres increases. The slope of linear fitting of $1/T_1$ vs. C@Gd₂O₃ concentration yields a longitudinal relaxivity (r_1) value of $4.23 \pm 0.13 \text{ mM}^{-1} \cdot \text{s}^{-1}$, which is comparable to commercial Gd chelates (e.g., Gd-DTPA, Gd-DOTA; $4\text{--}5 \text{ mM}^{-1} \cdot \text{s}^{-1}$) [6]. However, PL results show that the C@Gd₂O₃ spheres can be also utilized as an effective dual-mode contrast agent for MRI and optical imaging. In addition, the thin carbon coating on the surface of Gd₂O₃ spheres can protect tissues from leaching of free toxic Gd³⁺ metal ions.

4 Conclusions

In summary, hydrothermal method was used to produce carbon coated Gd₂O₃ particles. The morphology, structural, and optical properties of the synthesized samples were investigated by TEM, XRD, UV-Vis, and PL spectroscopy. MRI relaxivity studies of the C@Gd₂O₃ particles showed high longitudinal relaxivity ($r_1 = 4.23 \pm 0.13 \text{ mM}^{-1} \cdot \text{s}^{-1}$) of water protons, suggesting that C@Gd₂O₃ particles can be used as an efficient T_1 “positive” contrast agent. The thin carbon layer coated on the surface of Gd₂O₃ particles produced bright blue fluorescence, suggesting that the prepared C@Gd₂O₃ particles could serve as a bimodal agent for MRI and optical imaging.

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